

An Increased Capacity for Adrenal DHEA Release is Associated with Decreased Avoidance and Negative Mood Symptoms in Women with PTSD

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We recently found increased adrenal cortisol responses to adrenocorticotrophic hormone (ACTH)_{1–24} and increased pituitary ACTH and adrenal cortisol responses to corticotropin-releasing factor in premenopausal women with chronic post-traumatic stress disorder (PTSD) compared to healthy nontraumatized subjects. This pattern of hypothalamic–pituitary–adrenal axis (HPA) hyper-reactivity has been previously seen in healthy individuals treated with the antiglyucocorticoid mifepristone. We therefore investigated whether endogenous plasma levels of antiglyucocorticoids such as dehydroepiandrosterone (DHEA) and progesterone were increased in premenopausal women with PTSD at baseline or in response to adrenal activation by ACTH_{1–24}. The study revealed that DHEA responses to 250 µg ACTH_{1–24} were increased in 13 PTSD subjects compared to 13 healthy nontraumatized subjects, while DHEA levels were generally increased in the PTSD subjects compared to seven healthy traumatized subjects. Cortisol responses to ACTH_{1–24} were also higher in the women with PTSD, while progesterone levels and responses were not different among the three groups. In addition, among the PTSD subjects, the peak change in DHEA in response to ACTH_{1–24} was negatively correlated with the total Clinician Administered PTSD Scale score, while the peak DHEA to cortisol ratio was inversely associated with negative mood symptoms measured by the Profile of Mood States scale. This work suggests that an increased capacity for DHEA release in response to extreme adrenal activation may influence the pattern of HPA axis adaptation to extreme stress, as well as mitigate the severity of PTSD and negative mood symptoms in premenopausal women with PTSD.

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INTRODUCTION

We recently reported increased adrenal cortisol responses to intravenously administered adrenocorticotrophic hormone (ACTH)_{1–24} in premenopausal women with chronic post-traumatic stress disorder (PTSD) compared to healthy nontraumatized subjects (Rasmusson *et al*, 2001). In addition, we observed increased pituitary ACTH and adrenal cortisol responses to intravenously administered

ovine corticotropin-releasing factor (CRF) in the PTSD group. While these findings are inconsistent with some previous studies showing low (Mason *et al*, 1986, Yehuda *et al*, 1990, 1993, 1995) or the same cortisol output (Kosten *et al*, 1990; Baker *et al*, 1999; Mason *et al*, 2002) in PTSD compared to healthy subjects, they are consistent with other studies finding relatively increased urinary free cortisol output in premenopausal women (Rasmusson *et al*, 2001; Lemieux and Coe, 1995; Friedman *et al*, 2001), mixed-sex samples of children (De Bellis *et al*, 1999) or adults (Maes *et al*, 1998), and male combat veterans (Pitman and Orr, 1990) with PTSD. Since high levels of cortisol may contribute to alterations in the structure and function of brain areas and neurotransmitter systems thought to be involved in the mediation of PTSD symptoms (Sapolsky, 1985, 1986; McEwen, 2000; Schulkin *et al*, 1998; Grundemann *et al*, 1998; Lupien *et al*, 1998, 1999; Newcomer *et al*,

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1999; Gold *et al*, 2002), we decided it would be important to investigate the possible mechanisms underlying increased cortisol reactivity in premenopausal women with PTSD.

Increased ACTH and cortisol responses to CRF administration have been observed in animals and humans treated with the antiglucocorticoid RU486 (Van Haast *et al*, 1996; Bertagna *et al*, 1994). Facilitation of HPA axis reactivity by RU486 is thought to be mediated by antagonism of glucocorticoid negative feedback in the brain and pituitary, a resulting increase in ACTH release, and trophic effects of ACTH on the adrenal gland. Thus, we wondered whether endogenous, peripherally released steroids with antiglucocorticoid activity might play a role in enhancing pituitary and adrenal responsiveness in our female subjects with PTSD.

The endogenous steroids, dehydroepiandrosterone (DHEA) and progesterone, have been shown to have antiglucocorticoid activity in several tissues, including the brain (Chader and Reif-Leher, 1972; Kaiser *et al*, 1979; Bohus and DeKloet, 1981; Giesen and Beck, 1982; Daynes *et al*, 1990; Blauer *et al*, 1991; Browne *et al*, 1992; Araneo *et al*, 1993). DHEA and progesterone exert antiglucocorticoid effects at the glucocorticoid type II receptor via mechanisms distinct from classical receptor antagonism (Chou and Luttmann, 1998; Morfin and Starka, 2001), while progesterone acts as a classical antagonist at mineralocorticoid (type I glucocorticoid) receptors (reviewed in Turner, 1997). Consistent with the antiglucocorticoid characteristics of these steroids, ACTH responses to psychological stress have been shown to increase after DHEA treatment in postmenopausal women (Kudielka *et al*, 1998) and dexamethasone suppression of plasma cortisol levels has been shown to be reduced during the luteal phase of the menstrual cycle when progesterone levels are high (Altemus *et al*, 1997). DHEA and its sulfated metabolite, DHEAS, also positively modulate NMDA receptor function and antagonize GABAA receptor-mediated chloride ion flux (Baulieu and Robel, 1998)—effects that may contribute to increased HPA axis responses (Barbaccio *et al*, 2001).

DHEA is secreted episodically and synchronously with cortisol in response to fluctuating ACTH levels (Rosenfeld *et al*, 1971). In addition, the administration of CRF and ACTH has been shown to increase plasma DHEA and progesterone levels (Parker *et al*, 1985a,b; Genazzani *et al*, 1998). We therefore hypothesized that premenopausal women with chronic PTSD would have increased DHEA and/or progesterone levels at baseline or in response to adrenal activation. This would be of interest not only from a neuroendocrine point of view, but also because DHEA(S) has been shown protect the brain from the negative impact of stress (Kimonides *et al*, 1998; Bastianetto *et al*, 1999; Kaminska *et al*, 2000; Karishma and Herbert, 2002; Zhang *et al*, 2002), while the pharmacological properties of these neuroactive steroids or their metabolites might allow them to modulate PTSD symptoms (Rupprecht and Holsboer, 1999). We therefore measured plasma DHEA and progesterone levels in premenopausal women with chronic PTSD compared to healthy nontraumatized (NTC), and trauma-exposed (TC) subjects before and after the intravenous administration of ACTH_{1–24}.

METHODS

Subjects

In all, 41 subjects were recruited via community and clinic advertisements in Connecticut and Boston to participate in the study. The protocol was approved by the VA Connecticut and VA Boston Healthcare System Human Studies Subcommittees; written informed consent was obtained from each subject.

Subjects were screened for current or significant past medical illness and illicit drug use via medical history, physical examination, and laboratory tests. None had identifiable endocrine syndromes and only one healthy subject reported irregular menses. All subjects were free from psychotropic medications for months to years or had never been treated with such. Subjects abstained from other medications, alcohol, and illicit drugs for at least 4 weeks before testing, except for two PTSD and two TC subjects who used steroid hormone contraception. Two PTSD, one TC, and two NTC subjects smoked 1/2–1 pack of cigarettes per day.

DSM-IV PTSD Criterion A1/A2 trauma (American Psychiatric Association, 1994) exposure was assessed by use of general and early trauma inventories (ETI) (Bremner *et al*, 2000) (Table 1). The A1 criterion requires that a person experiences, witnesses or confronts an event or events involving actual or threatened death or serious injury or threat to the physical integrity of self or others. The A2 criterion requires that a person's response involve intense fear, helplessness, or horror. To qualify as a Criterion A2 trauma, we required that physical or sexual trauma ascertained by the ETI be scored as 'very' or 'extremely' distressing and disruptive in at least two of three domains of function (emotional, interpersonal, or school performance).

Healthy NTC subjects were evaluated psychiatrically using the non-patient edition of the Structured Clinical Interview for DSM-IV (SCID-NP) (First *et al*, 1995a); subjects with a current Axis I diagnosis were excluded, except for one subject with a simple phobia. PTSD and TC subjects were evaluated using the SCID-P (First *et al*, 1995b); subjects with current or past psychotic disorders or bipolar disorder were excluded from participation. Current co-morbid Axis I diagnoses are presented in Table 1.

Past psychiatric diagnoses among the PTSD subjects included major depression ($n = 11$), panic disorder ($n = 2$), substance abuse or dependence (> 6 years before participation, $n = 6$), simple phobia ($n = 2$), eating disorder (> 10 years before participation, $n = 1$), dysthymia ($n = 1$), OCD (washing and cleaning only, $n = 1$), and generalized anxiety disorder ($n = 1$). Two TC subjects had remote histories of single episode major depression. One NTC subject had one episode of past major depression and one a remote history of bulimia.

The PTSD, TC, and NTC subjects did not differ significantly by age: $F(2, 38) = 1.59$, $p = 0.22$ (PTSD: 36.1 ± 2.2 years, TC: 33.1 ± 4.3 years, NTC: 30.5 ± 2.1 years) or weight: $F(2, 38) = 0.65$, $p = 0.53$ (PTSD: 162.7 ± 10.4 lbs, TC: 150.9 ± 10.2 lbs, NTC: 150.6 ± 6.2 lbs). The PTSD subjects were of mixed Caucasian ($n = 13$), African American ($n = 1$), and Hispanic ($n = 1$) ethnicity. TC subjects were of mixed Caucasian ($n = 4$), African American ($n = 2$),

Table 1 Current Psychiatric Diagnoses and DSMIV Criterion A1, A2 Traumas

Group (CAPS score ^a)	Co-morbid diagnoses	Trauma (age in years)
PTSD (74)	MDD	Physical abuse (2–10) ^b Sexual abuse (4–5) Rape (13, 14) ^b Domestic violence (23–25)
PTSD (60)	None	Sexual assault (6) ^b Witnessed atrocities (7)
PTSD (59) ^c	None	Physical abuse (2–14) Sexual abuse (1–14) ^b Domestic violence (18–34) Rape (28)
PTSD (21)	None	^b Raped twice (12)
PTSD (18)	GAD	Physical abuse (8–13) ^b Rape (20)
PTSD (33) ^c	GAD Eating disorder NOS	Physical abuse (12–17) Rape (13, 17) Attempted rape (19) ^b Physical assault (29)
PTSD (35)	None	^b ~ Physical abuse (2–14) Sexual abuse (11–13)
PTSD (33)	None	^b Physical abuse (8–18) ^b Sexual abuse (15–18)
PTSD (47)	Dysthymia	Rape (8) ^b Domestic violence (33)
PTSD (64)	None	Sexual abuse (1–7) ^b Parent's death (7)
PTSD (70) ^c	None	^b Gang rape (16) Sexual abuse (2–16)
PTSD (20)	None	Physical abuse (13–15) ^b Rape (14)
PTSD (36)	MDD	^b Witnessed parent's sudden death (10) ^b Rape (19), near-drowning (21)
PTSD (31)	None	Rape (15) ^b Abduction and rape (22)
PTSD (74)	Dysthymia	Physical abuse (2–12) Sexual abuse (2–18) ^b Gang rape (22)

Table 1 (Continued)

Group (CAPS score ^a)	Co-morbid diagnoses	Trauma (age in years)
TC (14) ^c	Simple phobia	^b Motor accident (31)
TC (12)	None	Physical abuse (2–17) ^b Rape (34)
TC (0)	None	^b Witnessed domestic violence (9) Sexual abuse (12)
TC (3)	None	^b Witnessed murder (15)
TC (0)	None	^b Combat trauma (25)
TC (0)	None	^b Witnessed shooting (16)
TC (0)	None	^b Near fatal allergic reaction (18)

CAPS: Clinician Administered PTSD Scale; GAD: generalized anxiety disorder; MDD: major depressive disorder; OCD: obsessive compulsive disorder; MJ: marijuana; PTSD: post-traumatic stress disorder subject; TC: healthy trauma-exposed subject; NTC: healthy nontraumatized subject.

^aOne-week CAPS (Clinician Administered PTSD Scale) scores were ascertained at the time of the ACTH stimulation test. Subjects were instructed to report PTSD symptoms related to any of the reported A1/A2 traumas.

^bIndicates DSMIV A1, A2 traumas identified as most disturbing currently.

^cIndicates subjects (three PTSD, one TC) for whom childhood trauma was assessed using only the general trauma inventory that contained general questions regarding exposure to childhood physical or sexual abuse. ~ Indicates that trauma was verified by state records.

and Asian ($n=1$) descent. NTC subjects were of mixed Caucasian ($n=12$), African American ($n=1$), Asian (1), Askenazi Jewish ($n=1$), native Greek ($n=1$), Hispanic ($n=1$), native Russian ($n=1$), and subcontinental Indian ($n=1$) ethnicity. Years of education differed significantly, but not substantially among groups: $F(2, 38) = 4.46$, $p < 0.02$ (PTSD: 14.2 ± 0.54 , TC: 14.9 ± 0.99 , NTC: 16.5 ± 0.52 years). All subjects but one with PTSD had completed high school.

Menstrual Cycle Monitoring

Menstrual cycle phase at the time of the ACTH stimulation test (Table 2) was determined by use of a urine test kit to detect the mid-cycle luteinizing hormone (LH) surge (Clear Plan Easy, Whitehall Laboratories, Madison, NJ). Ovulation was confirmed by measurement of plasma progesterone levels 7–14 days later (Leibluft *et al*, 1994). In the data analyses, subjects on oral contraceptives were considered to be in the follicular phase.

ACTH Stimulation Test

Fifteen PTSD, 19 NTC, and seven TC subjects completed the ACTH stimulation test. Subjects were asked to fast for 4 h before testing. While many subjects fasted longer, the mean fasting times were not significantly different among the groups: $F(2, 27) = 1.98$, $p = 0.16$ (PTSD: 8.3 ± 1.7 h, TC: 12.9 ± 1.1 h, NTC: 11.4 ± 1.5 h). Subjects also refrained from

Table 2 Menstrual Phase During the ACTH_{1–24} Stimulation Test

	Follicular	Steroid contraception	Luteal
*Number of subjects in DHEA(S) analyses			
PTSD	8	3	2
NTC	7	0	6
TC	4	2	1
Number of subjects in cortisol analyses			
PTSD	8	3	2
NTC	8	0	7
TC	4	2	1
Number of subjects in progesterone and estrogen analyses			
PTSD	8	3	2
NTC	10	0	7
TC	4	2	0

smoking for 4–6 h prior to ACTH administration. ACTH_{1–24} (Cosyntropin, Organon Inc.) was administered intravenously 1 h after IV placement between 0800 and 1630 h, at a maximally stimulating dose of 250 µg. The average time of administration did not differ among groups: PTSD: 1125 h ± 30 min, range: 0915–1615 h; TC: 1053 h ± 37 min, range: 0930–1315 h; NTC: 1144 h ± 32 min, range: 0815–1345 h). Most of these subjects also participated in other tests designed to examine the HPA axis at different regulatory points. We thus allowed subjects to schedule their ACTH tests at their convenience since previous work by Dickstein *et al* (1991) had shown adrenal capacity to be unaffected by the timing of ACTH_{1–24} administration.

Blood samples were collected at –15, 0, +30, and +60 min relative to the ACTH_{1–24} infusion. They were spun immediately in a refrigerated centrifuge and plasma was stored at –70°C until hormone measurements were made. The Clinician Administered PTSD Scale (Blake *et al*, 1993) (CAPS-One Week Version; PTSD and TC subjects only) and Profile of Mood States scale (rated for the past week) (POMS; Educational and Industrial Testing Service, San Diego, CA) were obtained during the 30 min period after IV placement.

Plasma Hormone Measurements

Plasma cortisol was measured in 35 subjects, while DHEA and DHEA-S were measured in 33 subjects in the Yale Mental Health Clinical Research Laboratory. Plasma progesterone and estrogen were measured in 36 subjects in the Laboratory of Molecular Pathophysiology at the Mood and Anxiety Disorders Program, National Institute of Mental Health. Plasma cortisol was measured using an antibody-coated tube radioimmunoassay (RIA) with an inter-assay coefficient of variation (CV) of 8.2% and intra-assay CV of <5% (DiaSorin, Inc., Stillwater, MN). Plasma DHEA and DHEA-S were measured using RIA kits supplied by Diagnostic Systems Laboratories (Webster, TX). Plasma

DHEA was determined with a sensitivity (detectability) of 0.02 ng/ml, a cross-reactivity with DHEA-S of 0.02%, and intra- and inter-assay CVs of 8 and 11%, respectively. Plasma DHEA-S was measured with a sensitivity of 2 µg/dl and intra- and inter-assay CVs of 11 and 15%, respectively. Progesterone and estrogen were measured by solid-phase RIA (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA) with a sensitivity of 0.5 ng/ml. The inter-assay CV varied from 5 to 12%; the intra-assay CV was <5%.

As indicated above, the subject numbers vary among the hormone analyses. The cortisol assays were run first. Plasma was not available for two NTC subjects when the DHEA(S) assays were run. Additional NTC subjects were completed by the time the plasma progesterone and estrogen assays were run, but we no longer had access to the lab that ran the cortisol and DHEA(S) analyses. Data from all subjects meeting the inclusion criteria and completing the ACTH study and hormone measurements were included in the data analyses.

Statistical Analyses

A random effects model was used to analyze repeated hormone measurements from the ACTH stimulation test. Diagnostic group, time, and menstrual phase were included as independent variables and a compound symmetric variance structure (CS) was used in the mixed model (Diggle *et al*, 1994). A high-order interaction term was removed from the model if the *p*-value for this term was larger than 0.1. Although Dickstein *et al* (1991) showed that cortisol levels measured at 30' and 60' after a maximally stimulating 250 µg ACTH dose are not affected by the time of testing, baseline cortisol and DHEA levels (Erosheva *et al*, 2000) are known to progressively decrease from early morning until late afternoon. Baseline DHEA(S) levels are also known to decrease over the age range of subjects in the study (Hornsby, 1995). Therefore, the time of the ACTH_{1–24} injection and subject age were used as covariates. If there was a statistically significant difference in the overall model, pair-wise comparisons were conducted for the PTSD *vs* NTC, and PTSD *vs* TC groups; a closed testing procedure was applied to the pair-wise models.

A one-way analysis of variance (ANOVA) was used to compare the baseline or peak cortisol and DHEA hormone values among groups. The time of the ACTH_{1–24} injection was used as a covariate for the baseline but not peak steroid level analyses (Dickstein *et al*, 1991); age was also used as a covariate for the DHEA(S) analyses.

Pearson correlations were performed between age and DHEA(S) levels. Since the symptom ratings scores were not distributed normally, Spearman correlations were performed between DHEA or cortisol measures and symptom ratings. Due to the high degree of colinearity among rated psychiatric symptoms, as well as among the hormone responses to ACTH stimulation, Bonferroni corrections may impose a high risk of Type II error. Therefore, significance was set at *p* < 0.05 for the main correlations between DHEA or cortisol measures in response to ACTH and the total CAPS or total POMS scores. Bonferroni corrections were used in correlations between DHEA or cortisol reactivity to ACTH and the CAPS subscales or

component symptoms. Descriptive statistics are expressed as group means with standard errors of the mean.

It should be noted that this is not a replication study; the sample of subjects in the cortisol analysis is not independent from that in our previously published paper (Rasmusson *et al*, 2001). Two healthy NTC subjects and the subjects in the TC group were studied subsequent to completion of the previous study. Their data were added to the cortisol data from the previous group, and the data from this larger sample were reanalyzed.

RESULTS

Plasma DHEA and DHEAS Responses to ACTH Administration

Analysis of the ACTH stimulation test results indicated that age had a significant effect on plasma DHEA levels: $F(1, 28) = 14.32$, $p < 0.0007$, and correlated negatively with baseline plasma DHEA levels: $r = -0.58$, $p < 0.0005$. There was no effect of menstrual phase on absolute DHEA levels or responses to $ACTH_{1-24}$; therefore, menstrual phase was removed from the model. As expected, plasma DHEA levels increased in response to $ACTH_{1-24}$ administration: $F(2, 59) = 39.04$, $p < 0.0001$ (Figure 1a). In addition, there was an effect of diagnosis: $F(2, 28) = 7.21$, $p < 0.004$, as well as a diagnosis by time interaction: $F(4, 59) = 2.96$, $p < 0.03$. *Post hoc* testing indicated that plasma DHEA levels were higher across all time points: $F(1, 22) = 12.87$, $p < 0.002$, and increased more in response to $ACTH_{1-24}$: $F(2, 47) = 4.93$, $p < 0.012$, in the PTSD group compared to the NTC group. Plasma DHEA levels were higher across all time points in the PTSD group compared to the TC group: $F(1, 16) = 7.82$, $p < 0.013$, but there was no significant difference between these two groups in the DHEA response to $ACTH_{1-24}$: $F(2, 36) = 1.40$, $p = 0.26$. Baseline plasma DHEA levels were not significantly different among the PTSD, TC, and NTC groups: $F(2, 27) = 1.66$, $p = 0.21$. There were, however, differences in peak DHEA levels among the three groups: $F(2, 27) = 9.34$, $p < 0.0009$. Peak DHEA levels after $ACTH_{1-24}$ were higher in the PTSD group than in either the NTC group: $F(1, 27) = 16.67$, $p < 0.0005$, or the TC group: $F(1, 27) = 9.51$, $p < 0.005$.

We also measured the effects of ACTH on the sulfated metabolite of DHEA. As expected, age influenced plasma DHEAS levels during the ACTH test: $F(1, 28) = 11.46$, $p < 0.003$, and correlated negatively with baseline DHEAS levels: $r = -0.52$, $p < 0.003$. While DHEAS levels increased in response to $ACTH_{1-24}$ administration: $F(2, 60) = 7.08$, $p < 0.002$, DHEAS responses to ACTH were similar among the three groups: $F(4, 60) = 0.46$, $p = 0.77$. We did not find a significant difference among the three groups in DHEAS levels either: $F(2, 28) = 1.43$, $p = 0.26$; however, the study is underpowered and we cannot rule out Type II error.

Plasma Cortisol Responses to ACTH Administration

Consistent with previous work by Kirschbaum *et al* (1999), menstrual phase had no effect on plasma cortisol levels or responses to ACTH, and was removed from the model. As expected, plasma cortisol increased in response to $ACTH_{1-24}$: $F(2, 63) = 222.41$, $p < 0.0001$ (Figure 1b). There

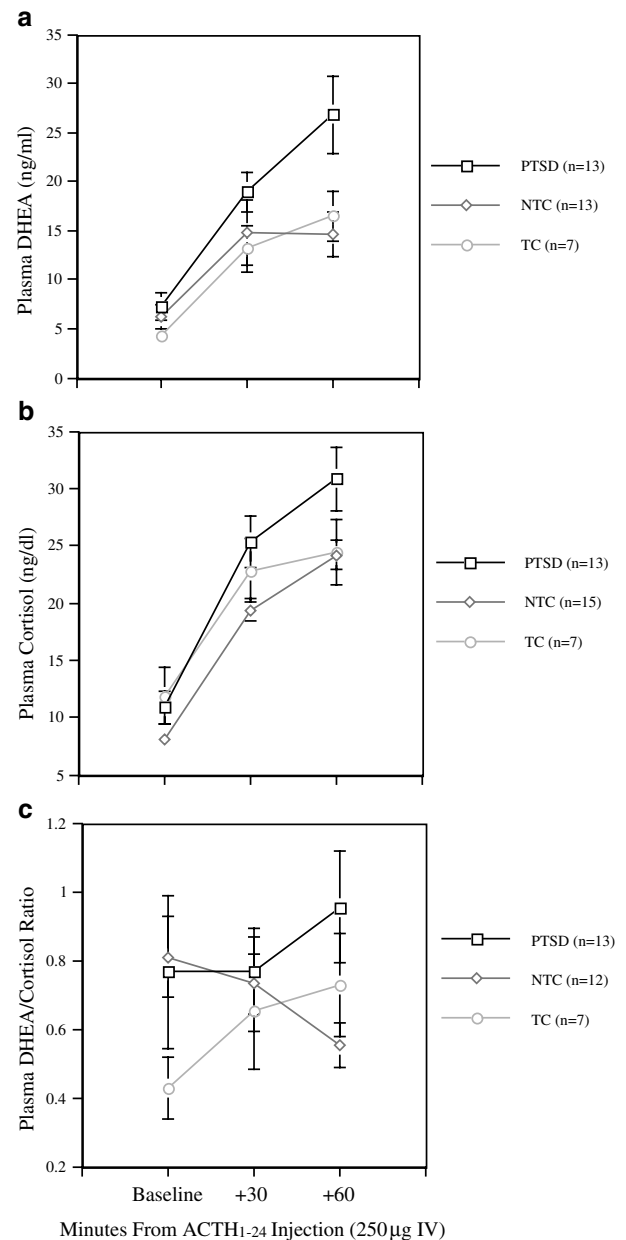


Figure 1 Plasma DHEA (a), cortisol (b), and DHEA/cortisol ratios (c) in response to a maximally stimulating 250 μ g dose of adrenocorticotrophic hormone ($ACTH_{1-24}$). PTSD: post-traumatic stress disorder; NTC: nontraumatized comparison; TC: trauma-exposed comparison subjects.

was also a significant diagnosis by time interaction: $F(4, 63) = 3.01$, $p < 0.025$. *Post hoc* pair-wise testing indicated that cortisol increased more in the PTSD than the TC group after $ACTH_{1-24}$: $F(2, 36) = 4.30$, $p < 0.03$. Consistent with our previous report (Rasmusson *et al*, 2001), plasma cortisol levels were higher across all time points in the PTSD compared to the NTC group: $F(1, 24) = 4.38$, $p < 0.05$, but there was no significant difference between these groups in the slope of the cortisol response to $ACTH_{1-24}$: $F(2, 51) = 2.11$, $p = 0.13$. Baseline cortisol levels measured prior to $ACTH_{1-24}$ administration were not significantly different among the PTSD, TC, and NTC groups: $F(2, 27) = 1.63$, $p = 0.22$.

The Ratio of Plasma DHEA to Cortisol

There was a significant diagnosis by time effect on the ratio of DHEA to cortisol: $F(2, 60) = 5.95$, $p < 0.005$ (Figure 1c). *Post hoc* analyses indicated that the DHEA/cortisol ratio increased similarly in response to ACTH₁₋₂₄ administration in the PTSD and TC groups (effect of time: $F(1, 36) = 8.11$, $p < 0.008$; diagnosis by time effect: $F(1, 36) = 0.43$, $p = 0.51$). The NTC group ratio behaved differently than the TC group (diagnosis by time effect: $F(1, 37) = 13.21$, $p < 0.0009$) and the PTSD group (diagnosis by time effect: $F(1, 47) = 6.33$, $p < 0.02$). There were no group differences in the baseline ($F(2, 27) = 1.84$, $p = 0.18$) or 30 min ($F(2, 27) = 1.10$, $p = 0.35$) DHEA/cortisol ratio. Group differences in the DHEA/cortisol ratio emerged 60 min after ACTH₁₋₂₄ administration: $F(2, 27) = 6.91$, $p < 0.004$, at which time the PTSD group ratio was significantly greater than the NTC group ratio ($p < 0.04$), while the TC group ratio fell between these groups.

Progesterone and Estrogen Responses to ACTH Administration

As expected, progesterone levels varied across the menstrual cycle and were about an order of magnitude higher in the luteal compared to the follicular phase in all groups: $F(1, 29) = 68.24$, $p < 0.0001$. Progesterone levels appeared to change in response to ACTH₁₋₂₄: $F(2, 58) = 5.14$, $p < 0.009$, but there was a significant menstrual phase by time interaction: $F(2, 58) = 12.39$, $p < 0.0001$. Consistent with the work by Krut and Rolland (1982), increases in progesterone after ACTH stimulation were detectable during the follicular phase: $F(1, 45) = 132.93$, $p < 0.0001$, but not during the luteal phase: $F(1, 15) = 0.10$, $p = 0.76$. There were no differences among the three diagnostic groups in baseline progesterone levels: $F(2, 26) = 1.38$, $p = 0.27$, or responses to ACTH₁₋₂₄: $F(4, 56) = 0.69$, $p = 0.61$.

Plasma estrogen levels were not different between menstrual phases: $F(1, 34) = 0.8$, $p = 0.38$, and did not change in response to ACTH₁₋₂₄ administration: $F(2, 67) = 1.51$, $p = 0.23$. There were also no differences among the three diagnostic groups in estrogen levels: $F(2, 31) = 0.17$, $p = 0.84$, or reactivity to ACTH₁₋₂₄: $F(4, 65) = 0.76$, $p = 0.55$.

Correlation of Clinical Symptoms with Plasma Hormone Responses

As expected, there was a positive correlation between the peak change in cortisol and peak change in DHEA after ACTH₁₋₂₄ administration among all subjects: $r = 0.48$, $p < 0.006$. The peak change in cortisol in response to ACTH₁₋₂₄ correlated positively and significantly with total POMS score: $r = 0.41$, $p < 0.03$, whereas the peak change in DHEA did not: $r = 0.22$, $p = 0.26$.

Within the PTSD group, the peak change in DHEA correlated negatively with the total CAPS score: $r = -0.57$, $p < 0.04$ (Figure 2a). This relationship appears to have been carried largely by a significant negative correlation between DHEA reactivity and Criterion C avoidance symptoms: $r = -0.70$, $p < 0.008$ (Figure 2b). Relationships between

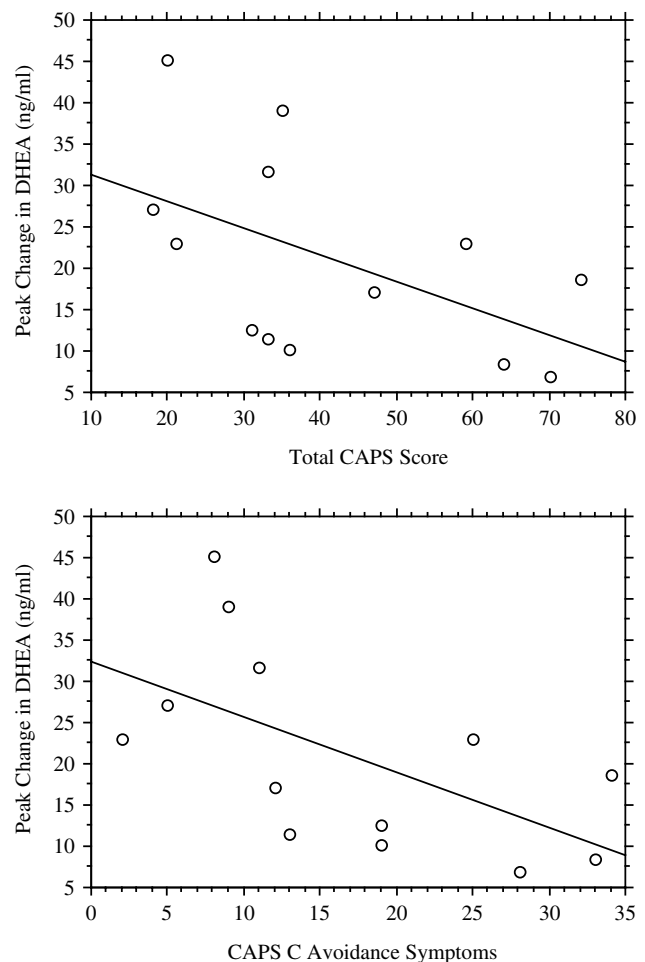


Figure 2 Scattergrams of the correlations between the peak change in plasma DHEA levels in response to 250 μ g ACTH₁₋₂₄ and: (a) total CAPS symptoms (Spearman $r = -0.57$, $p < 0.04$), (b) CAPS C avoidance symptoms (Spearman $r = -0.70$, $p < 0.008$), and (c) CAPS D hyperarousal symptoms (Spearman $r = -0.53$, $p = 0.06$).

DHEA reactivity and Criterion D hyperarousal symptoms: $r = -0.53$, $p = 0.06$ (Figure 2c), or Criterion B re-experiencing symptoms: $r = -0.19$, $p = 0.53$, were not significant. Of note, the peak change in DHEA correlated negatively, though not necessarily significantly, with each PTSD symptom comprising the Criterion B, C, and D clusters, except for 'difficulty falling or staying asleep' ($r = 0.52$, $p < 0.08$). Without inclusion of the sleep disturbance item in the total Criterion D symptom score, the correlation between Criterion D symptoms and the peak change in DHEA was strong and significant: $r = -0.81$, $p < 0.0009$.

In addition, within the PTSD group, there was a negative correlation between the mean DHEA/cortisol ratio after ACTH₁₋₂₄ and the POMS scores: $r = -0.63$, $p < 0.04$. There were no significant correlations between the peak change in cortisol in response to ACTH and total CAPS scores, summed Criterion B, C, or D scores, or total POMS scores in the PTSD subjects. However, the peak change in cortisol did correlate negatively, strongly, and significantly with 'decreased interest and participation in everyday activities': $r = -0.80$, $p = 0.001$.

DISCUSSION

In this study, we used a standard ACTH_{1–24} stimulation test to assess the maximum capacity of the adrenal gland for cortisol, DHEA, and progesterone release in premenopausal women with PTSD compared to healthy nontraumatized and trauma-exposed comparison subjects. This dose of ACTH elicits cortisol responses that equal or exceed those associated with extreme naturalistic stressors with the potential to cause PTSD (reviewed in Morgan *et al*, 2000). We found adrenal DHEA release to be increased in the women with PTSD compared to the healthy nontraumatized subjects, while the PTSD subjects had higher DHEA levels across all time points compared to the healthy trauma-exposed subjects. These findings are consistent with those of Lemieux and Coe (1995) showing an increase in 24-h urinary 17-ketosteroid metabolites of DHEA and DHEAS in premenopausal women with PTSD. They also may be consistent with two studies showing increased morning 'baseline' DHEA(S) levels in PTSD, since morning DHEA and cortisol levels reflect the reactivity of the adrenal gland to large pulses of endogenous ACTH. Spivak *et al* (2000) found high morning plasma DHEA and DHEAS levels, as well as a trend for an increase in plasma cortisol levels in Israeli males with combat-related PTSD. Sondergaard *et al* (2002) observed increased DHEAS levels among Kosovo refugees that developed PTSD but decreased DHEAS levels in refugees that developed PTSD and co-morbid depression. In that study, DHEAS levels were observed to increase over a 9-month period in association with increasing PTSD symptoms, particularly sleep disturbance. While we did not observe significant differences in plasma DHEAS levels between the traumatized subjects that did and did not develop PTSD in the current study, we cannot rule out Type II error due to the relatively small number of TC subjects.

In the current study, we also found increased cortisol responses to ACTH in the PTSD compared to healthy traumatized subjects, and higher cortisol levels in general in the PTSD compared to nontraumatized subjects. These findings are consistent with previous 24-h urinary free cortisol studies in premenopausal women and post-adrenarchal girls with PTSD, all of which have found increased cortisol output in the subjects with PTSD (Lemieux and Coe, 1995; Maes *et al*, 1998; De Bellis *et al*, 1999; Rasmusson *et al*, 2001; Friedman *et al*, 2001).

Our findings lead us to suggest that adrenally derived DHEA may contribute to an increase in the reactivity of the HPA axis in premenopausal women with PTSD. The antiglucocorticoid properties of DHEA may antagonize glucocorticoid negative feedback within the brain and pituitary, thereby facilitating CRF and/or ACTH release with a resulting increase in the adrenal capacity for cortisol release (Keller-Wood *et al*, 1984; Legradi *et al*, 1997; Heim *et al*, 2000; Rasmusson *et al*, 2001). This possibility is supported by studies in healthy volunteers of the glucocorticoid receptor antagonist mifepristone (RU486), which produces a pattern of pituitary and adrenal reactivity similar to that observed in our female subjects with PTSD (Van Haarst *et al*, 1996; Bertagna *et al*, 1994). This possibility is also supported

by studies that have shown: (a) increased ACTH reactivity to psychosocial stress in post-menopausal women treated for 2 weeks with DHEA (Kudielka *et al*, 1998), (b) increased ACTH and cortisol reactivity in heterozygote carriers of 21-hydroxylase deficiency, wherein cortisol precursors are shunted into the androgenic steroid pathways (Witchel *et al*, 1997; Merke *et al*, 1999; Witchel and Lee, 1998), and (c) increased CRF-induced ACTH release in depressed post-adrenarchal, pre-pubertal children with PTSD living in adverse environments (Kaufman *et al*, 1997). In addition, DHEA(S) positively modulates NMDA receptor function and antagonizes GABA_A receptors (Baulieu and Robel, 1998; Rupprecht and Holsboer, 1999), effects that may contribute to increased HPA axis responses (Barbaccio *et al*, 2001).

The current study does not support a role for adrenally derived progesterone in mediating differences in HPA axis responsivity between women with and without PTSD. However, it is important to note that the current study was not designed to determine whether progesterone derived from the ovary might play such a role. This will be important to assess in the future, since studies in healthy women have demonstrated increased ACTH and cortisol reactivity during the luteal compared to the follicular phase of the menstrual cycle (Kirschbaum *et al*, 1999; Altemus *et al*, 2001; Roca *et al*, 2003; Schmidt *et al*, 2002), effects that may be attributable to progesterone (Quinkler *et al*, 2003; Schmidt *et al*, 2002). It is also possible that antiglucocorticoid effects of progesterone play a role in differentiating patterns of HPA axis adaptation to stress between premenopausal women and other groups such as men, post-menopausal women, and pre-pubertal children. In addition, the conversion of DHEA to DHEAS, a metabolite that lacks antiglucocorticoid activity but that has more potent activity at NMDA and GABA_A receptors, appears to be inhibited by estrogen and facilitated by testosterone (Neslter, 1996). Thus, sex- as well as reproductive status-related roles for DHEA and progesterone in regulating HPA axis responsivity may help explain why the only studies finding low or similar urinary cortisol output in PTSD subjects have been those containing men alone or post-menopausal women (Mason *et al*, 1986, 2002; Yehuda *et al*, 1990, 1993, 1995; Kosten *et al*, 1990; Baker *et al*, 1999).

Negative Association between DHEA Responses and PTSD Symptoms and Negative Mood in PTSD

Interestingly, despite the association between PTSD and high-stimulated DHEA levels in the current study, there was a negative correlation between DHEA reactivity and severity of PTSD symptoms within the PTSD group, suggesting that DHEA may protect against the development of avoidance as well as hyperarousal symptoms. A role for DHEA as a resilience factor in PTSD is thus consistent with recent observations in military personnel of a negative correlation between DHEAS/cortisol ratios and dissociation and a positive correlation between DHEAS/cortisol ratios and military performance during extreme training stress (Morgan *et al*, 2004). There are also several other clinical studies showing a negative association between plasma DHEA(S) levels and depressive symptoms, and/or a positive

correlation between DHEA(S) levels and vigor or general well-being (Yaffe *et al*, 1998; Goodyer *et al*, 1998; Cruess *et al*, 1999; Heinz *et al*, 1999; Michael *et al*, 2000; Young *et al*, 2002). In addition, DHEA administration has been shown to treat depressive symptoms in individuals with major depression or adrenal insufficiency (Wolkowitz *et al*, 1999; Arlt *et al*, 1999), to reduce anxiety, depression, and negative symptoms in individuals with schizophrenia (Strous *et al*, 2003), and to mitigate depressive behavioral responses in genetically bred high- but not low-anxiety rats (Prasad *et al*, 1997).

Molecular and Neuropharmacological Effects of DHEA with Relevance to PTSD

In this context, it is important to appreciate that peripherally derived DHEA(S) is thought to be the only source of brain DHEA(S), since the biosynthetic pathway previously thought responsible for brain DHEA production functions only under nonphysiological conditions (Compagnone and Mellon, 2000). Within the brain, region-specific metabolism of DHEA may ultimately control the nature of DHEA effects on cognition and behavior (Rose *et al*, 1997). For instance, 7-hydroxylated metabolites of DHEA have been shown to interfere with the nuclear uptake of activated glucocorticoid receptors in neurons of the hippocampus (Morfin and Starka, 2001). This mechanism accords with the proposed anti-glucocorticoid properties of DHEA and may confer neuroprotection in this brain region. For example, DHEA protects against excitatory amino acid- and oxidative stress-induced damage, restores cortisol-induced decrements in long-term potentiation, regulates programmed cell death, and promotes neurogenesis in the hippocampus (Kimonides *et al*, 1998; Bastianetto *et al*, 1999; Kaminska *et al*, 2000; Karishma and Herbert, 2002; Zhang *et al*, 2002). Further research will be required to specifically determine whether and how DHEA may exert antiglucocorticoid effects in other brain regions of relevance to PTSD, such as the pituitary, hypothalamus, amygdala, and cortex. This is important since such effects may translate into behavioral alterations: genetic downregulation of brain glucocorticoid receptors has been shown to reduce anxiety-related behaviors in mice (Kellendonk *et al*, 2002).

It is also important to note that DHEA is a precursor for testosterone and estrogen, steroids with independent potent effects on brain, the HPA axis, and behavior (McEwen, 2002; Viau, 2002). In addition, pharmacological effects of DHEA or DHEAS may influence the PTSD phenotype. As Spivak *et al* (2000) suggested, DHEA(S) may potentiate activation of monoamine systems during stress—and thereby contribute to the consistently observed phenomenon of noradrenergic hyper-reactivity in PTSD (Southwick *et al*, 1999). In addition, DHEA may antagonize the capacity of cortisol to contain some aspects of the stress response (Munck *et al*, 1984), perhaps explaining how noradrenergic hyperreactivity can coexist with high as well as low cortisol output in PTSD. Facilitation of NMDA receptor function by DHEA(S) may also influence the development of PTSD. Activation of NMDA receptors in the amygdala has been shown to facilitate the formation as well as extinction of conditioned fear responses (Walker and Davis, 2002).

Increased DHEA(S) responses to extreme stress, could thereby enhance the development of traumatic memories as well as contribute to their extinction during naturalistic or treatment-based trauma re-exposure (Jaycox *et al*, 1998).

Factors that Influence DHEA Production and Release by the Adrenal Gland

Production of DHEA by the adrenal reticularis is a species-specific phenomenon; rodents have little circulating DHEA and the pattern of DHEA secretion differs between human and non-human primates (Neslter, 1996). In humans, plasma DHEA levels are high at birth due to the presence of the adrenal fetal zone, drop shortly thereafter, and remain low until adrenarche (age 6–8 years). DHEA levels then rapidly increase, continue to climb through adolescence, peak in the second to third decade, and then progressively decline with further aging (Hornsby, 1995). Thus, blunted DHEA responses to traumatic stress prior to adrenarche or later in life may limit the degree to which the HPA axis can upregulate in response to stress. A lack of neuroprotection by DHEA during periods of life when DHEA levels are low may contribute to toxic effects of stress on the brain.

In the current study, disparities in the frequency, severity, duration, and developmental timing of DSMIV criterion A trauma exposure between the traumatized groups with and without PTSD lead one to hypothesize that increased DHEA and cortisol reactivity may simply result from cumulative stress exposure or exposure to extreme stress earlier in life (eg, see Heim *et al*, 2002; Rinne *et al*, 2003). However, a simple effect of trauma severity or developmental timing of trauma on DHEA reactivity is not consistent with our observation of a negative correlation between DHEA reactivity and PTSD symptom severity. Other factors must also be involved.

Other proposed modulators of DHEA(S) levels during extreme stress include insulin (Bernton *et al*, 1995), interleukin 6 (Path *et al*, 1997; Judd *et al*, 2000), and B-adrenergic receptor activation (Oberbeck *et al*, 1998). In addition, the current study and the work of Oberbeck *et al* (1998) suggest that extreme activation of the adrenal gland may result in saturation of the adrenal glucocorticoid synthetic pathway, with shunting of steroid precursors into androgen pathways. Such an effect may be magnified in individuals with functional polymorphisms of genes that code for adrenal enzymes involved in cortisol synthesis (Witchel *et al*, 1997; Jacobs *et al*, 1999).

Study Limitations

Due to the relatively low number of subjects in the trauma control group, there is inadequate statistical power to rule out significant differences in DHEA reactivity between the TC and PTSD group. We also might have expected high morning 'baseline' DHEA levels in the PTSD group; however, only about half of the subjects were studied in the morning, possibly limiting our power to detect such an effect. Variability in the time of the ACTH test does not, however, alter the confidence with which we can interpret the differences in DHEA and cortisol reactivity among

groups. Not only did we match and covary for the time of ACTH_{1–24} administration across groups, but Dickstein *et al* (1991) demonstrated that 30' and 60' cortisol responses to a maximally stimulating ACTH dose do not differ between morning and late afternoon. There was also variability in the length of fasting among participants. However, there is no evidence in the literature to suggest that baseline cortisol levels vary with fasting over the interval spanned in this study. In addition, glucose effects on plasma cortisol reactivity are thought to be mediated centrally via CRF (Gonzalez-Bono *et al*, 2002; Heptulla *et al*, 2001). Adrenal steroid responses to ACTH_{1–24} administration do not involve CRF.

Another limitation, as well as strength, of the current study is the inclusion of only two PTSD subjects with concurrent major depression. Epidemiological data suggest that major depression co-morbid with PTSD tends to constitute more severe PTSD (Breslau *et al*, 2000), in part because several avoidance and hyperarousal symptoms of PTSD (poor concentration, irritability, poor sleep, decreased interest and participation in activities, and restricted range of affect) overlap with symptoms of major depression. The current findings as well as those of Sondergaard *et al* (2002) suggest that a group of more severely affected PTSD subjects with co-morbid major depression might show lower DHEA levels or reactivity.

It is also not clear from this cross-sectional study whether individual patterns of DHEA and cortisol output in response to adrenal activation are trait or state characteristics. Indeed, 11 PTSD subjects in the study had *past* major depressive episodes. It will be important to learn whether increased cortisol and DHEA reactivity in persons with PTSD predisposes to such episodes and/or whether this pattern is maintained or changes during depressive episodes and subsequent treatment (eg, see Heuser *et al*, 1998). The current study also does not allow us to discern whether acute and brief or more prolonged surges in DHEA are needed to affect glucocorticoid receptor function or exert effects at GABA and NMDA receptors. Our data suggest that cortisol and DHEA increases after extreme activation of the adrenal gland may persist for some time in PTSD subjects—thus research looking at the time course of these responses will be important.

And finally, while this study suggests that the adrenal release of DHEA, a compound with antiglucocorticoid activity, is increased in premenopausal women with PTSD and may interfere, for example, with glucocorticoid negative feedback within the HPA axis, the latter has not been directly demonstrated. Previous studies using the dexamethasone suppression test (DST) have shown enhanced (Stein *et al*, 1997) or normal (Lipschitz *et al*, 2003) glucocorticoid negative feedback in premenopausal women with PTSD, or have contained too few female subjects to give reliable results (Grossman *et al*, 2003; Rinne *et al*, 2002)—especially since menstrual phase influences DST outcome (Altemus *et al*, 1997) and was not controlled in any of these studies. Thus, it will be important in the future to investigate whether DHEA modulates glucocorticoid negative feedback at the level of the pituitary in premenopausal women with PTSD. In addition, studies in animals may be used to investigate a role for DHEA in modulating

glucocorticoid negative feedback at higher levels of the central nervous system.

CONCLUSION

The current study suggests that an increased capacity for DHEA release in response to extreme adrenal activation may be protective and/or mitigate PTSD symptoms and negative mood in persons with PTSD. The results of this study should therefore spur us to examine how a shift toward androgen production, possibly influenced by genetic predisposition, as well as other biological and developmental factors, influences the pattern of neuropsychiatric and behavioral sequelae, as well as the neuroendocrine profile that develops in response to extreme stress. In addition, it will be critical to more fully understand the adaptive as well as possible maladaptive features of this pattern of neuroendocrine system adaptation (McEwen, 2000; Fabian *et al*, 2001). Hopefully, such work will allow us to develop new treatments for PTSD as well as new methods to identify and protect individuals at greater risk for PTSD in advance of anticipated extreme stress exposure.

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